Clinal variations of discriminative meristic characters of sea bass, *Dicentrarchus labrax* (Moronidae, Perciformes) populations on Tunisian coasts

by

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ABSTRACT. - Morphological variation of eight groups of samples (317 individuals) of Mediterranean Sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) collected throughout northern and eastern-south Tunisian coasts was investigated using seven meristic characters. Tunisian coasts are important to study as they are considered as a transitional area between western and eastern Mediterranean basins. Average comparisons and Discriminant Function Analysis showed, for two characters, the number of lateral line scales and gillrakers number, clusters allowing the distinction between samples collected in the eastern Mediterranean basin and those caught in the western basin. The comparison of the averages for these two characters with those reported in other geographical locations (Atlantic: Morocco; Golfe du Lion, Annaba and Gulf of Gabès) highlights a decreasing cline for the number of gillrakers and an increasing one for the number of lateral line scales, from the Atlantic Ocean to the western basin of the Mediterranean and then to the Eastern basin. These results suggest that the variation of these two meristic characters is related to the gradual evolution of physicochemical characteristics in these transitional areas. Moreover, these findings seem to be consistent with the existing pattern of genetic differentiation in this area.

RÉSUMÉ. - Variation clinale de caractères méristiques discriminants des populations du loup commun, *Dicentrarchus labrax* (Moronidae, Perciformes) des côtes tunisiennes.

La variabilité morphologique de huit lots d'échantillons (317 individus) du loup commun, *Dicentrarchus labrax* (Linnaeus, 1758), récoltés le long des côtes tunisiennes (Nord et Sud-Est), a été étudiée en se fondant sur sept caractères méristiques. Ces côtes sont importantes à étudier puisqu'elles sont considérées comme une zone de transition entre les deux bassins méditerranéens. La comparaison des moyennes ainsi que l'analyse factorielle discriminante ont montré, au niveau de deux caractères, nombre d'écailles de la ligne latérale (Ec) et nombre de branchiospines (Bsp), des regroupements permettant la distinction entre les échantillons collectés dans le bassin méditerranéen oriental et ceux qui ont été pris dans le bassin occidental. La comparaison des moyennes, pour ces deux caractères, avec celles qui ont été rapportées dans d'autres localités géographiques (Atlantique : Maroc ; Golfe du Lion, golfe d'Annaba et golfe de Gabès) met en évidence un cline décroissant pour le caractère nombre de branchiospines et croissant pour celui qui est relatif au nombre des écailles de la ligne latérale, en allant de l'Atlantique au bassin occidental de la Méditerranée puis au bassin oriental. Ces résultats montrent que la variabilité de ces deux caractères méristiques (Bsp et Ec) reflète l'évolution graduelle des conditions physico-chimiques du milieu marin le long de ces zones de transition. De plus, elle semble en accord avec le patron de différenciation génétique existant au niveau de cette région atlanto-méditerranéenne.

Key words. - Moronidae - *Dicentrarchus labrax* - MED - Tunisian coasts - Morphology - Clines - Meristic counts - Transitional area.

The Sea bass, *Dicentrarchus labrax* (Linnaeus, 1758) is a species belonging to the Mediterranean-Atlantic fauna. The genus seems to have an Atlantic origin and its presence in the Mediterranean would only be the result of immigration most likely during the glacial phases (Quignard, 1978; Lemaire *et al*., 2005). Currently, the distribution of this species ranges from Scandinavian to Senegalese coasts in the Atlantic. It is also present in the Mediterranean and Black Seas (Fischer *et al*., 1987). The presence of *Dicentrarchus lab*rax in the Red Sea (Gulf of Aqaba) is presumably due to human activity (Golani and Lerner, 2007). This wide distribution of *D. labrax* can be attributed to the fact that the species is euryhaline and eurythermal. In addition, it has strong dispersive capacity since its planktonic larval phase lasts from 2 to 3 months (Pickett and Pawson, 1994). Moreover, the migratory behaviour of adults cannot be neglected as a potential cause of the species distribution (Barnabé, 1973; Pickett and Pawson, 1994).

Several studies were dedicated to the characterization of the genetic structure of Atlanto-Mediterranean populations of this species. The use of allozymes and mitochondrial markers has identified, as a first step, three genetically distinct areas: the North-East Atlantic Ocean, the western Mediterranean and the Eastern Mediterranean (Benharrat *et al*., 1983; Patarnello *et al*., 1993; Allegrucci *et al*., 1997; Caccone *et al*., 1997; Cesaroni *et al*., 1997). A first transition zone has been delimited between the Atlantic and western Mediterranean, at the Almeria-Oran front (Naciri *et al*., 1999;

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Lemaire *et al.*, 2005), as a result of the use of six microsatellite loci and a fragment of cytochrome b. The second area is located between the East and West Mediterranean basins that are separated by the Siculo-Tunisian strait (Bahri-Sfar *et al.*, 2000). These genetic differences were attributed to a gene flow disruption in marine environment. During various episodes of the Pleistocene, changes in sea level led to a fragmentation of habitats and, therefore, have altered the connections between populations, hence the establishment of a genetically determined barrier (Lemaire *et al.*, 2005). The conjunction of this barrier with a physical barrier for migration, like hydrological factors, may explain the limited exchanges and maintenance of genetic disjunction in these areas (Borsa *et al.*, 1997; Lemaire *et al.*, 2005).

The study of these transitional zones awakened a discussion not only about how these genetic differences are maintained in spite of the continuous distribution range of populations (Lemaire *et al.*, 2005) but also their implication in phenotypes and life history characteristics of these populations.

Significant differences in some meristic characters, like the number of vertebrae or gillrakers, have already been reported among these Mediterranean transitional zones (Barnabé, 1973; Quignard, 1978; Kartas, 1981). Quignard (1978) believes that these differences, at these areas, reflect a stock independence.

In this study, we propose to examine the variability of several meristic characters in eight Tunisian groups of samples of sea bass collected from the southern shores of the western and eastern Mediterranean basins. Tunisian coasts are considered as a transitional area between western and eastern Mediterranean basins. Data will be compared to those reported in other geographical locations (Atlantic: Morocco; Golfe du Lion, Gulfs of Annaba and Gabès). The morphological characterization of these populations would allow the establishment of some morphological relationships that reflect the geographical position and the nature of the exchanges in this transitional area.

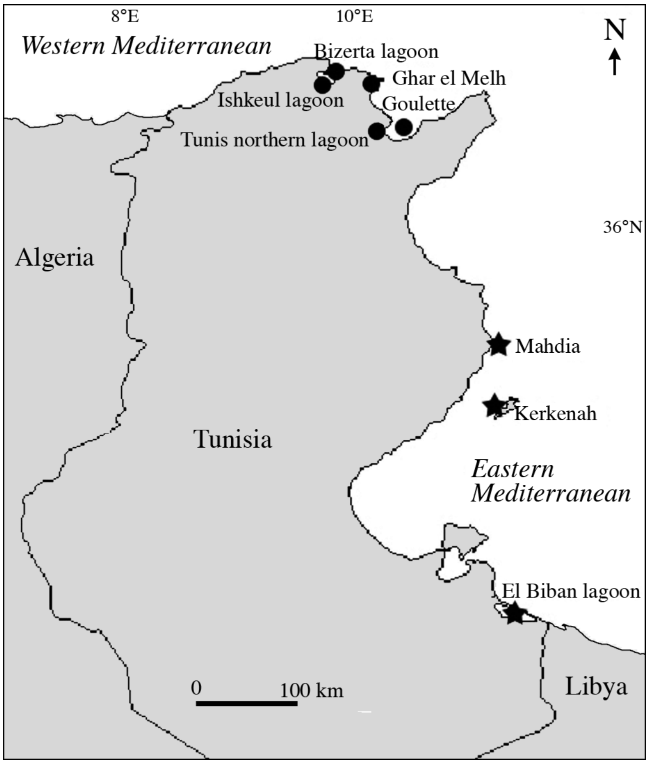

Figure 1. - Locations of sampling sites, along the Tunisian coasts. Sites in the eastern Mediterranean basin (★). Sites in the western Mediterranean basin (●).

MATERIALS AND METHODS

In order to study the morphological variability of Tunisian coast natural populations of common sea bass, three hundred seventeen individuals belonging to eight samples were collected from sites ranging from the northern (western basin) to the eastern-south (eastern basin) Tunisian coasts (Fig. 1, Tab. I).

Seven meristic characters have been counted: the number of hard rays of the 1st dorsal fin (D1), the number of soft rays at the 2nd dorsal fin (D2), anal (An), pectoral (Pct) and pelvic

Table I. - Characteristics of samples of Sea bass *Dicentrarchus labrax* collected on Tunisian coast.

Locality	Code	Sample sizes	Habitat	Mediterranean basin	Average total length (cm)	Total length range (cm)
Ishkeul lagoon	TISH	41	Lagoon	Western	23.47	21.4-30
Bizerta lagoon	TBIZ	55	Lagoon	Western	31.94	18.3-64
Gulf of Tunis (Ghar El Melh)	TGRM	36	Sea	Western	27.51	19.1-43.5
Gulf of Tunis (Goulette)	TGOU	47	Sea	Western	27.64	18.8-51
Tunis northern lagoon	TLNT	33	Lagoon	Western	36.67	21.4-49
Mahdia	TMAH	32	Sea	Eastern	36.15	30.6-42.5
Kerkenah	TKER	42	Sea	Eastern	30.24	30-36
El Biban lagoon	TBIB	31	lagoon	Eastern	40.78	31.4-52.3

(Plv) fins, the number of gillrakers on the first left branchial arch (Bsp) and the number of lateral line scales (El) (the rear edge of the head up to the level of hypural bone). The chosen characters have been used in other studies on morphological variation among different populations across the distribution range of this species.

To test the overall homogeneity or heterogeneity of variance averages, analysis of variance (ANOVA) was carried out using the software Statistica version 5.1 (1997). For each character, a comparison of average reunification of Duncun (Duncun, 1955) was carried out. This allowed the gathering of samples that yielded nonsignificantly different averages. This test was based on the calculation of the least significant differences (LSD) for groups of 2, 3, 4, …, p averages). Discriminant Function Analysis (DFA) was performed to identify axes which maximize the differences between the values of the dependent variable. This multivariate analysis was computed with the R software Version 2.2.0.

Table II. - Distributions of the seven meristic characters for each sample. n: Sample size; SD: Standard deviation. See figure 3 for abbreviations.

Samples		Bsp	Ec	D1	D2	Plv	An	Pct
Ishkeul lagoon	*n*	41	41	41	41	41	41	41
	Average	23.93	71.41	8.85	12.24	6	10.54	16.51
	SD	0.82	3.26	0.36	0.66	0	0.55	0.55
	Mode	24	71	9	12	6	11	16
	Range	(21-26)	(64-78)	(8-9)	(11-13)	-	(9-11)	(16-18)
Bizerta lagoon	*n*	55	55	49	49	49	49	49
	Average	23.55	70.65	8.96	12.57	6	10.98	16.78
	SD	1.23	2.87	0.2	0.5	0	0.38	0.62
	Mode	24	71	9	13	6	11	17
	Range	(20-26)	(63-78)	(8-9)	(12-13)	-	(10-12)	(15-18)
Ghar El Melh (Gulf of Tunis)	*n*	36	36	36	36	36	36	36
	Average	23.61	72.22	9	12.5	6	10.97	16.81
	SD	1.36	3.39	0.24	0.51	0	0.17	0.62
	Mode	24	73	9	13	6	11	17
	Range	(19-26)	(67-77)	(8-10)	(12-13)	-	(10-11)	(16-18)
Goulette (Gulf of Tunis)	*n*	47	47	45	45	45	45	45
	Average	23.94	73.87	8.91	12.4	5.98	10.93	16.56
	SD	1.03	3.73	0.36	0.5	0.15	0.45	0.59
	Mode	24	74	9	12	6	11	17
	Range	(21-26)	(65-81)	(8-10)	(12-13)	(5-6)	(10-12)	(16-18)
Tunis northern lagoon	*n*	33	33	33	33	33	33	33
	Average	23.12	71.67	8.94	12.55	6	10.85	16.94
	SD	1.52	2.97	0.24	0.67	0	0.36	0.5
	Mode	23	73	9	13	6	11	17
	Range	(19-26)	(68-76)	(8-9)	(11-13)	-	(10-11)	(16-18)
Mahdia	*n*	32	32	32	32	32	32	32
	Average	22.44	74.81	8.97	12.31	6	10.66	17.06
	SD	1.37	3.21	0.18	0.47	0	0.55	0.62
	Mode	23	74	9	12	6	11	17
	Range	(19-25)	(70-81)	(8-9)	(12-13)	-	(9-11)	(16-18)
Kerkenah	*n*	42	42	34	34	34	34	34
	Average	22.17	76.14	8.91	12.68	5.97	10.91	17.12
	SD	1.43	4.69	0.38	0.77	0.17	0.51	0.73
	Mode	22	82	9	12	6	11	17
	Range	(19-25)	(65-87)	(8-10)	(12-14)	(5-6)	(10-12)	(16-18)
El Biban lagoon	*n*	31	31	31	31	31	31	31
	Average	21.97	74.03	8.97	12.74	6	10.74	16.94
	SD	1.14	3.82	0.18	0.51	0	0.63	0.57
	Mode	23	74	9	13	6	11	17
	Range	(19-26)	(64-81)	(8-9)	(12-13)	(5-6)	(10-11)	(16-18)

RESULTS

Distributions of the seven studied meristic characters, for each collected sample, are given in table II. The comparison of variances shows a significantly heterogeneous averages for five variables (Bsp, Ec, D2, Pct, Ana) and reveals the great stability of the remaining two (D1 and Plv) (Tab. III). However, among variables assessing heterogeneous averages, only two (Bsp and El) show an evident and consistent discrimination between locations after running the average reunification of Duncun (Fig. 2). The total number of gillrakers (Bsp) significantly discriminates the samples according to their geographic areas (northern and eastern-south Tunisian coasts). A first group includes eastern-south samples (Mahdia, Kerkenah and lagoon El Biban) while the other consists of samples from the northern coasts (Fig. 2). Similarly, typology is very interesting insofar as it allows a distinction between northern and eastern-south samples for the number of lateral line scales. However, the rendered result remains imperfect since a northern sample: the Goulette (Gulf of Tunis) was grouped with the eastern south group.

79.6% of the total variance is explained by the first three axes of Discriminant Function Analysis (DFA). The DF1, which holds 52.6% of the total variability, is defined by two characters: the number of gillrakers and the number of lateral line scales (Fig. 3A). Spatial projection of class populations on the factorial plane defined by the first two axes (DF1 and DF2) showed the segregation of the eastern-south samples that were rendered on the negative side of the axis1 and the northern samples that were projected on the positive side of factor 1 (Fig. 3B).

DISCUSSION

The characterization of geographic populations is mainly based on the usage of genetic markers which have usually showed interesting and precise results. This particularly true in the case of Sea bass (Benharrat *et al.*, 1983; Patar-

Table III. - ANOVA on meristic counts in *Dicentrarchus labrax* samples. NS: not significant; *: Significant at 5% threshold; F: Fischer-Snedecor variable.

Variable	n	Average	Min.	Max.	Std. Dev.	F	P	Significance
Gillrakers (Bsp)	317	23.17	19	26	1.43	15.78	< 0.001	*
Lateral line scales (Ec)	317	72.99	63	87	3.95	12.32	< 0.001	*
Dorsal 1 (D1)	301	8.94	8	10	0.28	1.02	> 0.05	NS
Dorsal 2 (D2)	301	12.49	11	15	0.59	3.18	< 0.01	*
Pelvic (Plv)	301	5.99	5	6	0.08	0.82	> 0.05	NS
Anal (Ana)	301	10.83	9	12	0.48	4.83	< 0.001	*
Pectoral (Pct)	301	16.81	15	19	0.63	5.06	< 0.001	*

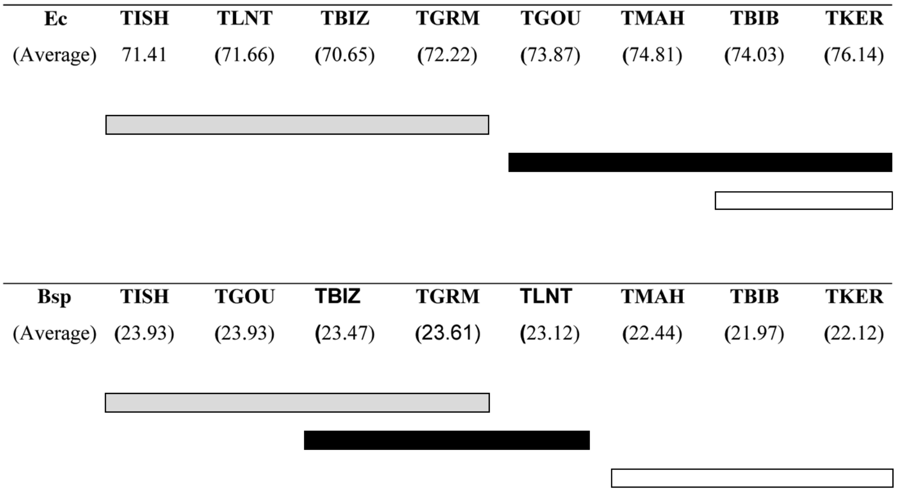
Ec TISH TLNT TBIZ TGRM TGOU TMAH TBIB TKER
(Average) 71.41 (71.66) (70.65) (72.22) (73.87) (74.81) (74.03) (76.14)
Bsp TISH TGOU TBIZ TGRM TLNT TMAH TBIB TKER
(Average) (23.93) (23.93) (23.47) (23.61) (23.12) (22.44) (21.97) (22.12)

Figure 2. - Comparison of sample averages of discriminative characters (Duncun test at 5% threshold). Samples related by trait have not statistical different averages. Ec: number of lateral line scales; Bsp: number of gillrakers on the first left branchial arch. See table I for abbreviations.

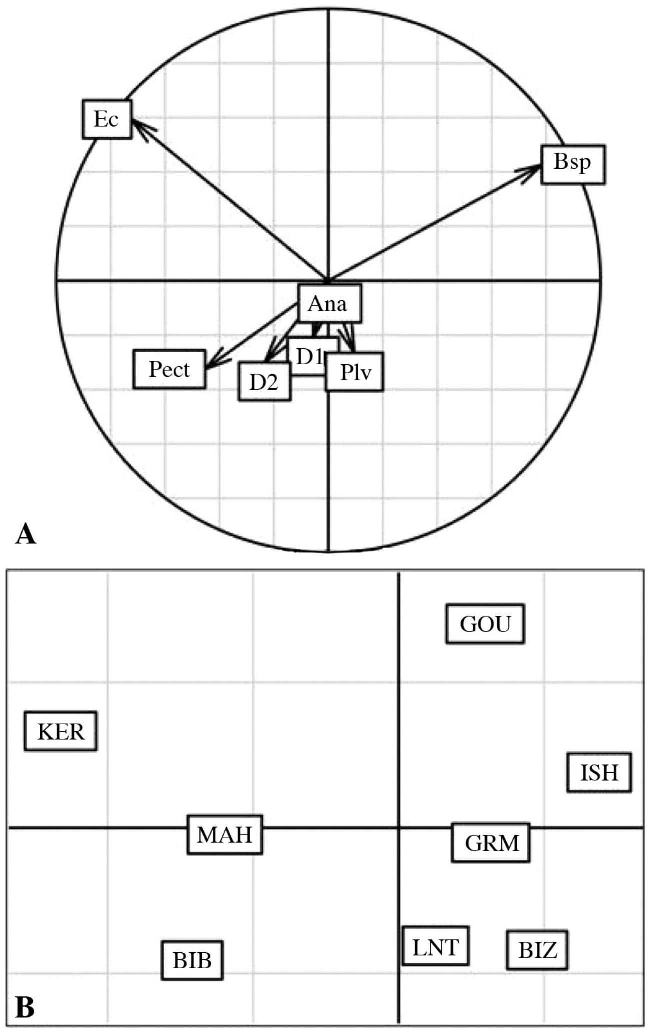

Figure 3. - Discriminant Function Analysis (DFA) of seven meristic characters in eight samples of *Dicentrarchus labrax* on DF1×DF2 plan. **A**: Correlating circle: Variable correlation coefficient on discriminant functions DF1 and DF2. **B**: DFA class scores. Sample codes: see table I.

Ana: Anal; Bsp: Gill rakers; D1: Dorsal 1; D2: Dorsal 2; Ec: Lateral line scales; Pct: Pectoral; Plv: Pelvic fins.

nello *et al.*, 1993; Allegrucci *et al.*, 1997; Caccone *et al.*, 1997; Cesaroni *et al.*, 1997; Naciri *et al.*, 1999; Bahri-Sfar *et al.*, 2000; Castilho and Ciftci, 2005; Lemaire *et al.*, 2005). Nevertheless, morphological characters have been commonly used in fish biology to delineate fish stocks (Roldan *et al.*, 2000; Ismen, 2001; Afonso-dias *et al.*, 2002; Langerhans *et al.*, 2003; Maltagliati *et al.*, 2003; Silva, 2003; Salini *et al.*, 2004; Turan, 2004; Ergüden and Turan, 2005). Phenotypic variations are known to exhibit both environmental and genetic components (Lindsey, 1988; Parsons, 1992; Robinson and Wilson, 1996; Cabral *et al.*, 2003). Therefore, variations of these different characters can be a first step conducting to genetic population structure investigations (Kara and Fehri, 1997; Turan, 1999).

The present study demonstrates a variation of two meristic characters (Bsp and El) of common Sea bass samples caught along the Tunisian coasts according to their geographic origins. These variables seem to set apart the samples of the eastern-south sector from those of the northern sector. The variation of gillrakers number, along the Tunisian coasts, seems to follow a decreasing cline from the northern to the eastern-southern sector. On the other hand, the variation in the number of lateral line scales seems to have an increasing cline in the same direction. The comparison of the obtained data for these two characters with those retrieved in other geographic localities: Atlantic coasts of Morocco (Gravier, 1961), Golfe du Lion (Barnabé, 1973), Gulf of Annaba in Algeria (Kara and Fehri, 1997) and Gulf of Gabès in Tunisia (Bouaïn, 1977), clearly shows the existence of the above mentioned clines (Tab. IV, Fig. 4). Thus, the variation of gillrakers number and that of lateral line scales matched respectively with a decreasing and an increasing clines from the Atlantic toward the western Mediterranean basin and then to the eastern one.

Barnabé (1973) has proposed to use these two characters to discriminate populations of western Mediterranean and Atlantic. We have just demonstrated that these two meristic characters can both discriminate Atlantic Sea bass populations from those of the western Mediterranean and populations of the latter area from those of the eastern Mediterranean. In this context, it is worth mentioning that the number of lateral line scales is considered as one of the main criteria for distinguishing between populations. This character has allowed the distinction of Atlantic and Mediterranean *Atherina* and highlighted significant differences between *Atherina* populations of the Tunisian coast (Trabelsi *et al.*, 2000; Trabelsi *et al.*, 2002a, 2002b). It is also one of the main characters used to describe Bluefish, *Pomatomus saltatrix,* population's characteristics in the Eastern Mediterranean (Turan *et al.*, 2006)

On a large scale, these meristic variations are usually accompanied by the observation of clines. Regarding the number of gillrakers, a decreasing clinal variation was also emphasized for *Sardina pilchardus* (Kartas, 1981). However, for the Hake, *Merluccius merluccius* (Maurin, 1968) and the *Sardinella aurita* (Kartas, 1981), the cline was observed to increase from the Atlantic to the western Mediterranean. Depending on whether a cline is decreasing or increasing, these variations seem to reflect the gradual variation of physical and chemical conditions of the marine environment especially in these two transitional areas (Gibraltar Strait and Siculo-Tunisian Strait). It would be interesting to know the ecological particularities of these areas in order to determine the ambient parameters that are important in the variation of the gillrakers number or the lateral line scales number for a given species. Environmental factors, particularly salinity,

Table IV. - Averages of the two discriminative variables in different geographic localities.

Geographic area	Gillraker number averages	Lateral line scales number averages
Atlantic coasts of Morocco (Gravier, 1961)	25.63	68.21
Golfe-du-Lion (Barnabé, 1973)	23.46	72.66
Gulf of Annaba (Kara and Fehri, 1997)	23.76	71.39
Ishkeul lagoon	23.93	71.41
Bizerta lagoon	23.55	70.65
Gulf of Tunis: Ghar El Melh	23.61	72.22
Tunis northern lagoon	23.12	71.67
Gulf of Tunis : Goulette	23.94	73.87
Mahdia	22.44	74.81
Kerkenah	22.17	76.14
Gulf of Gabès (Bouaïn, 1977)	22.3	73.4
El Biban lagoon	21.97	74.03

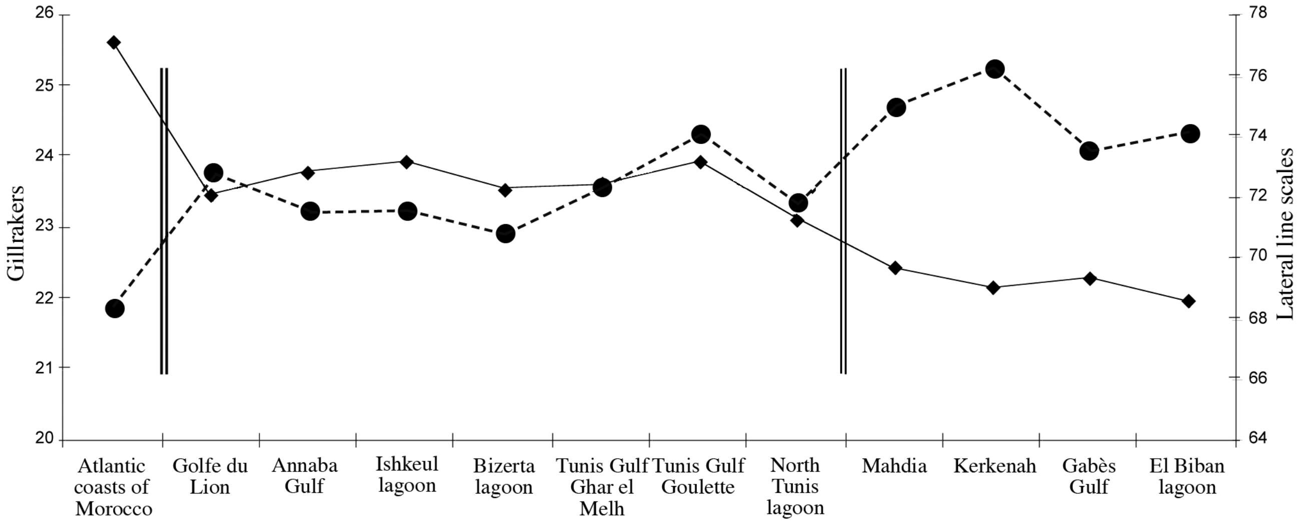

Figure 4. - Variation of number of gillrakers and lateral line scales from Atlantic ocean (Morocco: Gravier, 1961), western Mediterranean (Golfe du Lion, Sète: Barnabé (1973); Algerian coasts, Gulf of Annaba: Kara and Fehri (1997) and Northern Tunisian coasts: present study) to southern Tunisian coasts (Gulf of Gabès: Bouaïn (1977) and present study). — Gillrakers number; - - - Lateral line scales number.

would explain the observed clines (Barnabé, 1973; Quignard, 1978), since it gradually increases from the Atlantic Ocean to the western Mediterranean basin and thus to the eastern one (Millot *et al.*, 2006). The influence of water salinity on the number of gillrakers was reported on *Dicentrarchus labrax* (Loy *et al.*, 1999) and other species like *Fundulus heteroclitus* (Tay and Garside, 1972).

Other arguments have been reported to explain these gradual changes in gillrakers number, as selection pressures arising from the quality and the abundance of available food (Kiener and Spillman, 1969; Gross and Anderson, 1984) or the increase in fish size (Kartas, 1981). Nevertheless, this last argument cannot explain our results, given the lack of correlation between fish size and the number of gillrakers in this study.

Until now, it's assumed that variation of morphological characters and especially meristic ones have both environmental and genetic components (Lindsey, 1988; Parsons, 1992; Cabral *et al.*, 2003). This study has allowed us, through the variation of two meristic characters (Bsp and El), to differentiate western Mediterranean basin sea bass populations from those of the eastern Mediterranean basin. These results are consistent with those obtained, for the same samples used in this study, using six polymorphic microsatellites loci (Bahri-Sfar *et al.*, 2000). Indeed, samples from the North of Tunisian coasts genetically differ from those collected in the South-East-sector. The same northern samples belong to a homogeneous entity regrouping all genetic samples of the Western Mediterranean which are genetically different from those of Atlantic Ocean (Naciri *et al.*, 1999).

According to all these results, we can conclude that the genetic factor partly explains observed meristic discrimination of sea bass samples. Meristic differences between samples of the common sea bass from the two Mediterranean basins and the Atlantic reflect the genetic originality of populations belonging to these three geographic areas. This seems to be the result of restrictions of gene flows between them. Moreover, these local populations, from the same geographical area (belonging to a group of breeders, with a separate and well-defined reproduction area), seem to develop adaptations to the local environmental conditions by natural selection.

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